

# **Title: Influence of Acidosis on Cardiotonic Effects of Colforsin and Epinephrine: A Dose-Response Study**

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## Abstract

**Objective:** Acidosis produces a negative inotropic effect on cardiac muscle against which catecholamines and phosphodiesterase III inhibitors have limited therapeutic effects. This study was designed to evaluate effects of colforsin, which directly activates adenylate cyclase without beta-receptor activation, in isolated Langendorff rat hearts in a pH- and concentration-dependent manner.

**Design:** Experimental animal study.

**Setting:** A university laboratory.

**Participants:** Sprague-Dawley rats.

**Interventions:** Hearts were isolated and perfused with HEPES-Tyrode solution (pH 7.4) in the Langendorff preparation. The hearts were randomly assigned to control (pH 7.4), mild (pH 7.0), and severe (pH 6.6) acidosis groups (n = 8 each) and were continuously perfused with  $10^{-7}$  M,  $10^{-6}$  M, and  $10^{-5}$  M colforsin.

**Measurements and Main Results:** Left ventricular maximum rate of increase (LV dP/dtmax) were determined, and the concentration-response relationship were evaluated at each pH. Colforsin at  $10^{-6}$  M increased LV dP/dtmax from  $2592 \pm 557$  to  $5189 \pm 721$  mmHg/s ( $P < 0.001$ ) and from  $1942 \pm 325$  to  $3399 \pm 608$  mmHg/s ( $P < 0.001$ ) in the control and mild acidosis groups, respectively, while  $10^{-5}$  M colforsin significantly increased LV dP/dtmax even in the severe acidosis group. No significant difference was seen in LV dP/dtmax among the three groups after  $10^{-5}$  M colforsin infusion.

**Conclusion:** In contrast with catecholamines and other inodilators, colforsin at high concentration restores diminished cardiac contractility against severe acidosis to the extent similar to the physiological pH.

**Keywords:** acidosis, adenylate cyclase, colforsin, cyclic AMP

## Introduction

Acidosis produces a negative inotropic effect on cardiac muscle<sup>1-6</sup> and diminishes the inotropic effects of catecholamines<sup>7-11</sup> and of phosphodiesterase isozyme III (PDE III) inhibitor<sup>12</sup>. The inotropic effect of catecholamines and PDE III inhibitor results mainly from increased concentrations of intracellular cyclic adenosine monophosphate (cAMP) generated by stimulating beta-adrenergic receptors and by inhibiting PDE III, respectively. Although the cause of the diminished myocardial responses to catecholamines has not been completely elucidated, decreased cAMP production as a result of depressed adenylate cyclase activity<sup>7</sup>, decreased numbers of beta-adrenergic receptors<sup>7,8</sup>, decreased affinity for agonists<sup>9</sup>, decreased Ca<sup>2+</sup> sensitivity of myofilaments<sup>1</sup>, and reducing opening of the sarcoplasmic reticulum Ca<sup>2+</sup> release channel<sup>13</sup> might be involved.

Colforsin (NKH477;6-(3-dimethyl-aminopropionyl) forskolin hydrochloride) is a water-soluble forskolin derivative that increases cardiac output and the maximum rate of increase in left ventricular pressure (LV dP/dtmax) in patients with left ventricular systolic dysfunction<sup>14</sup>. Colforsin exerts positive inotropic action by directly activating adenylate cyclase and might therefore increase heart contractility in acidosis. However, the influence of acidosis on inotropic effects of colforsin has not been evaluated.

We hypothesized that colforsin **augments the** contractility of isolated rat heart **as a result of concomitant increase in cAMP** in severe acidosis. Accordingly, this investigation was designed to evaluate cardiotonic effects of colforsin in a concentration-dependent manner at various degrees of acidosis, and determined cAMP concentrations to elucidate its possible contribution in an isolated rat heart preparation.

## Methods

### Experimental animals

The Animal Research Committee at University of Tsukuba approved this study. Nine-week-old male Sprague-Dawley rats (Japan SLC, Shizuoka, Japan), weighing 280 to 360 g, were housed in stainless-steel cages and maintained at  $23.5 \pm 2.5^{\circ}\text{C}$ ,  $52.5 \pm 12.5\%$  humidity and a 14-h light and 10-h dark cycle with free access to food and water ad libitum.

### Langendorff heart preparation

One hundred and two male Sprague-Dawley rats aged 10 weeks were first assigned to either experiment 1 (hemodynamic measurements,  $n = 66$ ) or experiment 2 (cAMP measurement,  $n = 36$ ). In both experiments, rat hearts were similarly prepared and perfused with Langendorff apparatus. They were anesthetized with an intraperitoneal injection of pentobarbital sodium (80 mg/kg). The hearts were isolated *via* thoracotomy and the aorta was connected to a Langendorff apparatus (RADNOTI LLC, Monrovia, CA, USA). The hearts were perfused at constant flow of approximately 10 mL/min for several minutes and then were gravity perfused at a constant pressure with HEPES-Tyrodé solution ( $\text{pH} = 7.4$ ) at  $37.0 \pm 0.3^{\circ}\text{C}$  in a thermostatically controlled recirculating water bath (Harvard Apparatus Ltd, Edenbridge, Kent, UK). The HEPES-Tyrodé solution comprised (mM) 137.0 NaCl, 6.0 KCl, 1.0  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 1.8  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 5.5 Glucose, 10 HEPES, and NaOH titrated to the required pH, and bubbled with 100%  $\text{O}_2$ . A fluid-filled balloon was inserted into the left ventricle via an incision in the left atrium and inflated at 2 to 5 mmHg of left ventricular end-diastolic pressure during the initial stabilization period. After stabilization and baseline measurements, we measured coronary perfusion flow (CPF), coronary perfusion pressure (CPP), and left ventricular pressure (LVP). Measurements of CPP were made using a

pressure transducer at the aortic level. The LV dP/dtmax, determined by differentiating LVP waveform, was computed using AcqKnowledge™ software (BIOPAC Systems, Goleta, CA, USA). All pressure data are digitized using a 16-bit analog-digital converter, stored at a sampling rate of 1000 Hz in a computer, and subsequently analyzed offline. For real-time smoothing transformation of LVP, dP/dtmax and HR data, smoothing factor of three was used in our study, ie. an average was computed as the mean of three adjacent data of the moving “window” before moving on to the next data set. Ten values of CPP, LVP and LV dP/dtmax in each measurement period were averaged and reported as a representative of each heart at each pH and at each colforsin or adrenaline concentration. CPF was measured by collecting the perfusate draining from the perfused heart for a minute.

#### Experiment 1 (Figure 1)

Sixty-six rats were first assigned to either colforsin study (n = 24), adrenaline study (n = 24), or vehicle control (n = 18), and then, subsequently randomized to the control (pH = 7.4), mild acidosis (pH = 7.0), and severe acidosis (pH = 6.6) groups (n = 8 each for colforsin and adrenaline study, n=6 each for vehicle control). After 10 minutes of stabilization using HEPES-Tyrode solution of pH 7.4, LVP, CPP, and CPF were determined, and then the HEPES-Tyrode solution adjusted to the appropriate pH for each group was perfused continuously throughout the experiment. Ten minutes after the pH change, Langendorff hearts were continuously perfused with constant pressure with  $10^{-7}$  M,  $10^{-6}$  M, and  $10^{-5}$  M colforsin or with  $10^{-8}$  M,  $10^{-7}$  M, and  $10^{-6}$  M adrenaline for 10 minutes each. We measured LVP, CPP, and CPF 10 minutes after the pH change and at the end of each concentration of colforsin or adrenaline for 10 minutes. This time interval represented a peak contractile effect for all concentrations of cardiotonic drugs in our experiment (Figure 2). The concentration-



response relationships between these variables and colforsin ( $10^{-7}$  to  $10^{-5}$  M) or adrenaline ( $10^{-8}$  to  $10^{-6}$  M) were determined at each pH.

#### Experiment 2 (Figure 1)

The remaining 36 rats were randomly assigned to the following six groups: with  $10^{-5}$  M colforsin at pH 7.4 and at pH 6.6, with  $10^{-6}$  M adrenaline at pH 7.4 and at pH 6.6, and without cardiotoxic drugs at pH 7.4 and pH 6.6 ( $n = 6$  each). Ten minutes after connecting the Langendorff apparatus, hearts were perfused with pH 7.4 HEPES-Tyrodé solution for 10 minutes, and then, at either pH 7.4 or 6.6 throughout the experiment. The colforsin and adrenaline groups of hearts at both pH were continuously infused with colforsin ( $10^{-7}$ ,  $10^{-6}$ , and  $10^{-5}$  M) and adrenaline ( $10^{-8}$ ,  $10^{-7}$ , and  $10^{-6}$  M) for 10 minutes in a similar manner to that of the experiment 1, and then, the hearts were immediately frozen in liquid nitrogen ( $-45^{\circ}\text{C}$ ). Vehicle control groups received 30 minutes of saline infusion instead of colforsin or adrenaline at both pH, and then hearts were similarly frozen. The left ventricular muscles were separated, disrupted in 5 ml of 5% trichloroacetic acid using a digital homogenizer (Iuchi, Osaka, Japan) and centrifuged at  $2,000 \times g$  for 15 minutes at  $4^{\circ}\text{C}$ . The supernatants were washed four times with 5 ml of diethyl ether saturated with water. The upper ether layer was discarded after each wash. The remaining aqueous extract was dried under a stream of nitrogen at  $60^{\circ}\text{C}$ . The dried extracts were dissolved in 300  $\mu\text{l}$  of RPMI 1640 medium. Concentrations of cAMP were quantified using a dextran-coated charcoal radioimmunoassay (SRL, Inc., Tokyo, Japan). The sensitivity of this method (average binding ratio of 80 pmol/ml standard solution minus average binding ratio of 0 pmol/ml standard solution) is over 38%, detection limit is 0.62 pmol/ml, and coefficient of variation is 3.1 to 4.9%.

## Chemicals

Sodium pentobarbital (Nembutal injection), a product of Abbott Laboratories (Abbott Park, Chicago, IL, USA) was purchased from Dainippon Sumitomo Pharmaceutical Co. (Osaka, Japan), and adrenaline was purchased from Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Colforsin was kindly provided from Nippon Kayaku, Co., Ltd.

## Statistics

All data are expressed as mean  $\pm$  SD. Statistical differences in LV dP/dtmax and CPF were assessed using repeated-measures ANOVA followed by **unpaired *t*-test with** Scheffé's test to adjust for multiple comparisons, with a probability level of  $P < 0.05$  being significant. **Unpaired *t*-test was also used for comparison between variables with cardiotonic drugs at each pH and time- (vehicle-) control values.** Concentrations of cAMP between two pH's were compared using unpaired *t*-test with a probability level of  $P < 0.05$  being significant. All data were analyzed using Stat-View software (SAS Inc., Cary, NC, USA).

## Results

The CPP values remained stable from 62 to 63 mmHg throughout the experiment in all groups. The pH change decreased HR in the severe acidosis group from  $200 \pm 34$  to  $108 \pm 24$  bpm ( $P < 0.001$ ), but not in the control and mild acidosis groups (from  $218 \pm 38$  to  $213 \pm 43$  bpm, and from  $194 \pm 19$  to  $171 \pm 28$  bpm, respectively). Colforsin at  $10^{-5}$  M increased HR in the severe acidosis, mild acidosis, and control groups (to  $215 \pm 39$ ,  $235 \pm 31$ , and  $269 \pm 26$  bpm, respectively), but did not result in any significant differences among the three groups at  $10^{-5}$  M colforsin. Similarly, adrenaline  $10^{-6}$  M increased HR in the severe acidosis, mild acidosis, and control groups (from  $110 \pm 18$  to  $173 \pm 24$ , from  $172 \pm 26$  to  $235 \pm 35$ , and from  $163 \pm 16$  to  $231 \pm 45$  bpm, respectively), but did not result in any significant differences among the three groups.

Mild (pH 7.0) and severe (pH 6.6) acidosis groups developed significantly decreased LV dP/dtmax compared with pre-pH change (Figures 3). LV dP/dtmax at each pH were essentially unchanged without colforsin or adrenaline throughout the experiment. Therefore, LV dP/dtmax with colforsin or adrenaline at each concentration was also compared with these time control values without cardiotoxic drugs. Colforsin ( $10^{-6}$  M and  $10^{-5}$  M) significantly increased LV dP/dtmax compared with the post-pH change in all the groups. Although LV dP/dtmax values in the mild and severe acidosis groups at  $10^{-6}$  M colforsin were significantly less than those of the control group, such difference dissipates at  $10^{-5}$  M colforsin (Figure 4). The pH change also decreased CPF significantly in the severe acidosis group, but not in the mild acidosis group (Figure 5). CPF remained unchanged for the entire experiment after pH change without colforsin or adrenaline (time control, data not shown). All tested concentrations of colforsin increased CPF compared with the post pH change in the all the groups (Figure 5). At colforsin at  $10^{-5}$  M, no significant difference was found in LV dP/dtmax, CPF or HR among the three groups.

Adrenaline  $10^{-7}$  M and  $10^{-6}$  M significantly increased LV dP/dtmax compared with the post-pH change in the control and mild acidosis groups (Figure 6). In the severe acidosis group, LV dP/dtmax increased significantly only by  $10^{-6}$  M adrenaline. In contrast to colforsin, adrenaline at its maximum dose ( $10^{-6}$  M) did not restore LV dP/dtmax to the level similar to physiological pH (Figure 6). The pH change also decreased CPF significantly in the severe acidosis group, but not in the mild acidosis group (Figure 7). CPF increased significantly at all tested concentrations of adrenaline in the control and mild acidosis groups. In the severe acidosis group, however, CPF remained unchanged at all concentrations studied.

The hemodynamic status of all groups in the cAMP study (experiment 2) was similar to those of the experiment 1 (data not shown). Severe acidosis decreased the amount of cAMP, whereas colforsin  $10^{-5}$  M increased cAMP concentrations to the level similar to that of pH 7.4 (Figure 8). On the other hand, even though adrenaline  $10^{-6}$  M increased cAMP contents compared with no adrenaline at both pH 7.4 and 6.6, that of pH 6.6 was significantly less than that of pH 7.4.

## Discussion

This is the first study which demonstrated concentration-dependent changes in cardiotonic effects by colforsin on a background of various degrees of acidosis. The present findings show that colforsin restores diminished cardiac contractility by severe acidosis to the level similar to the physiologic pH in isolated rat hearts, which has never been reported in the past using catecholamines or PDE III inhibitors. Indeed, maximum cardiotonic response even with the largest dose of adrenaline could not be attained in our study. These results suggest greater relative inotropic potency of colforsin in the acidotic environment. Therefore, colforsin could be theoretically useful in clinical situations where other inotropic drugs have failed to restore contractility, when acidosis is rigid and severe. However, further in vivo studies are warranted to confirm its cardiotonic effects under various degrees of acidosis using clinically relevant concentrations of colforsin.

One may argue that the maximum contractile response was not attained using  $10^{-6}$  M adrenaline and larger adrenaline dose might have produced cardiotonic effects to an extent similar to that at physiological pH, as seen with the largest concentration of colforsin. In our pilot study<sup>15</sup>, however,  $10^{-5}$  M adrenaline resulted in frequent occurrence of ventricular ectopic contractions in more than half of the preparations, and stable hemodynamics could not be obtained for further analysis of LV dP/dtmax. On the other hand, unlike other cardiotonic drugs including catecholamines and PDE III inhibitors, colforsin has been shown to suppress digitalis- and adrenaline-induced arrhythmia in canine model<sup>16</sup>, and indeed, ventricular arrhythmia was not observed in our preparation even with the largest colforsin concentration. Although colforsin has been clinically available since 1999, similar hemodynamic profile as an inodilator to PDE III inhibitors and relatively longer half-life ( $t_{1/2\beta} = 1.9 \pm 0.7$  hour,  $t_{1/2\gamma} = 95.3 \pm 15.2$  hour) in actual surgical patients hampered its clinical popularity<sup>17</sup>. In in vivo canine preparation, colforsin has been reported to increase myocardial

oxygen consumption<sup>18</sup>, while small dose of colforsin appears to produce improved cardiac performance without increases in myocardial oxygen demand in humans after coronary artery bypass grafting. More importantly, its anti-arrhythmogenic property together with restored cardiotonic effect during severe acidosis suggests its superiority when use of other inotropic agents is limited for unsatisfactory inotropic effects due to severe acidosis or arrhythmogenic property<sup>19</sup>.

Colforsin at  $10^{-7}$  and  $10^{-6}$  M failed to demonstrate improved cardiac performance at pH 6.6 to a level equivalent to that of pH 7.4. In contrast,  $10^{-5}$  M colforsin restored LV dP/dtmax even at pH 6.6. These results indicate that the inotropic effects of colforsin are decreased in the severely acidotic heart, but irrespective of catecholamines and PDE III inhibitors increasing colforsin concentration negates the deleterious effect of acidosis on its cardiotonic effect. Acidosis depresses cardiac performance<sup>1-6</sup> and the inotropic effect of catecholamines<sup>7-11</sup>. Several factors are considered to play a role in diminished myocardial responses to catecholamines, such as reduction in the numbers of beta-adrenergic receptors<sup>7,8</sup>, reduced affinity for agonists<sup>9</sup>, depressed adenylate cyclase activity<sup>7</sup>, decreased cAMP production<sup>7</sup>, inhibition of calcium ion exchange<sup>20</sup>, and decreased affinity of calcium ions for myofilaments<sup>11, 21, 22</sup>. Decreased cAMP turnover is also important in the deleterious effect of acidosis on pharmacologic action of milrinone<sup>12</sup>. Because colforsin exerts positive inotropic action by directly activating adenylate cyclase, a reduction in the numbers and affinity of beta-adrenergic receptors would not have affected the inotropic property of colforsin. Although changing the pH from 7.4 to 6.6 decreased the amount of cAMP,  $10^{-5}$  M colforsin restored cAMP concentration at pH 6.6 to that similar to pH 7.4 in the present study, suggesting that an increase in myocardial cAMP concentration may, in part, account for the inotropic effects of colforsin in severe acidosis. Further studies are required to understand the

more precise mechanisms of restored inotropic effect of colforsin, such as calcium ion exchange and calcium affinity to myofilaments.

In our study, cardiotonic effects of colforsin under mild acidosis appear to be augmented at a concentration-dependent manner, though to a less extent, than those at physiological pH. Previous literature showed that mechanical function to isoproterenol during acidosis (pH 6.8) was not different from control condition (pH 7.4) in isolated arterially perfused heart of the newborn rabbits<sup>7</sup>. Similarly, decreasing cellular pH from 7.4 to 6.8 resulted in a decline in contractile amplitude to isoproterenol only by 20% in cultured myocardial cells from chick embryo ventricle<sup>8</sup>. In a canine in vivo preparation, on the other hand, significant and profound depressions of LV dP/dtmax in response to 2 and 5 µg/kg/min of milrinone under mild acidosis (pH 7.0) compared with the physiological pH were observed<sup>12</sup>. These discrepant observations of myocardial contractile responses to various cardiotonic drugs under relatively mild acidotic condition may arise from the differences in animals, drugs, as well as in experimental models.

We examined how acidosis affects the cardiotonic effect of colforsin up to  $10^{-5}$  M, which is approximately 100-fold greater than the clinically applied concentration<sup>23</sup>. Since the isolated heart model was used in this investigation, effect of colforsin on blood pressure and the vascular system could not be evaluated. Colforsin stimulates mainly cardiac adenylyl cyclase, and in part, other tissue adenylyl cyclases (lung, brain, and kidney) type II, III and V<sup>24</sup>. Colforsin produces systemic and pulmonary vasodilation and increases renal blood flow<sup>25, 26</sup>. These factors may contribute to decreasing afterload and preload in vivo, which may ultimately lead to hypotension. In addition, chronic exposure to a high concentration of colforsin might cause hypertrophy of the heart, myocardial fibrosis, and myocardial degeneration<sup>27-29</sup>. Therefore, further studies using an in vivo model are warranted to evaluate

the relationships between acidosis and colforsin in a situation similar to the actual clinical environment as well as short-term toxicity of colforsin before a large dose is recommended in clinical situations.

Study limitations include significant decreases in CPF during acidosis, and thus, the change in hemodynamics might have involved an effect of the decreased CPF, **especially with adrenaline**. However, colforsin  $10^{-7}$  and  $10^{-6}$  M in the severe acidosis resulted in significant increases in CPF with limited effects on LV dP/dtmax, suggesting that CPF was not a sole mechanism to explain depressed cardiac performance in severe acidosis. Secondly, we did not enzymatically confirm the absence of severe myocardial damage by the experimental preparation. Therefore, the possibility that severe hypoxic or mechanical injury to the myocardium at the time of isolation of rat hearts contributed to the altered hemodynamic responses to colforsin **and adrenaline** in the severe acidosis groups cannot be ruled out. However, similar hemodynamics (LV dP/dtmax and CBF) before the pH change among the three groups **as well as stable time control preparations** do not support the presence of severe myocardial damage in our preparation.

In conclusion,  $10^{-5}$  M colforsin increased the LV contractility of isolated Langendorff rat hearts at pH 6.6 to a level similar to that at pH 7.4, whereas  $10^{-6}$  M adrenaline did not produce cardiac contractility similar to the physiological level. As opposed to currently available cardiotonic drugs, increasing dose of colforsin restored depressed cardiotonic effect induced by severe acidosis without increasing the incidence of ventricular arrhythmia. Thus, colforsin may be considered as an inotropic agent of choice when severe acidosis limits hemodynamic management in clinical settings.



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## Figure legends

**Figure 1:** Experimental protocols for measuring hemodynamics (Experiment 1) and tissue cAMP contents (Experiment 2) with or without colforsin or adrenaline at pH 7.4, 7.0 and 6.6.

**Figure 2:** Temporal changes in left ventricular pressure and left ventricular dP/dtmax of a typical representative heart, showing depressions in these variables after introduction of pH 6.6 and enhancement of these variables by escalating doses of colforsin. Note that peak and stable responses are obtained within 10 min after initiation of a new pH as well as three concentrations of colforsin.

**Figure 3:** Changes in left ventricular dP/dt max (LV dP/dtmax) without colforsin or adrenaline at pH 7.4, 7.0 and 6.6. Data are shown as means  $\pm$  SD (n=6 each). LV dP/dtmax significantly decreased with pH change in the mild and severe acidosis groups, and remained depressed throughout the experiment. LV dP/dtmax values at each pH were essentially unchanged over time after pH change. \* $P < 0.05$  vs. pre-pH change.

**Figure 4:** Changes in left ventricular dP/dtmax (LV dP/dtmax) with or without colforsin at pH 7.4, 7.0 and 6.6. Data are shown as means  $\pm$  SD (n=8 each). LV dP/dtmax decreased with pH change in the mild and severe acidosis groups. Colforsin significantly increased dP/dtmax in all groups at  $10^{-6}$  M and  $10^{-5}$  M. \* $P < 0.05$  vs. pre-pH change.  $^{\dagger}P < 0.05$  vs. post-pH change and time (vehicle) control values at corresponding pH (Figure 3).  $^{\ddagger}P < 0.05$  vs. control (pH 7.4) group. No significant difference was found among the three groups at colforsin  $10^{-5}$ .

**Figure 5:** Changes in coronary perfusion flow (CPF) with or without colforsin at pH 7.4, 7.0 and 6.6. Data are shown as means  $\pm$  SD (n=8 each). Change in pH decreased CPF in the severe acidosis group. Colforsin significantly increased CPF in all group at all concentrations compared with the post-pH change and the time control values (data not shown). \* $P < 0.05$  vs. pre-pH change.  $^{\dagger}P < 0.05$  vs. post-pH change and time (vehicle) control values at corresponding pH. No significant difference was found among the three groups.

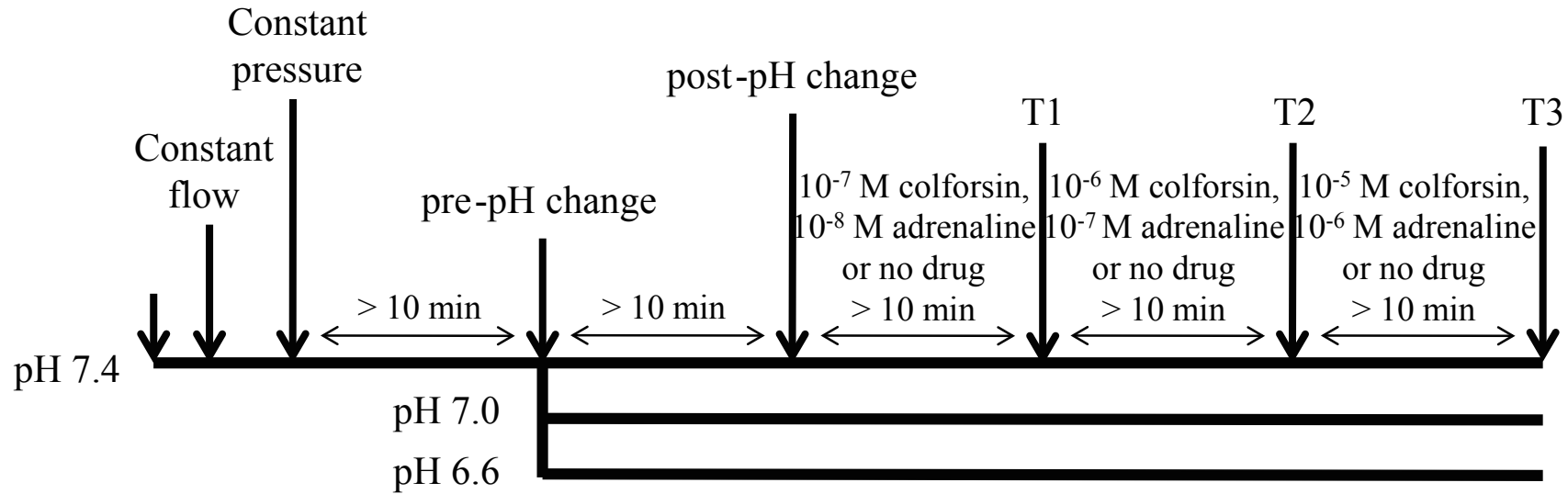
**Figure 6:** Changes in left ventricular dP/dtmax (LV dP/dtmax) with or without adrenaline at pH 7.4, 7.0 and 6.6. Data are shown as means  $\pm$  SD (n=8 each). LV dP/dtmax decreased with pH change in the mild and severe acidosis groups. Adrenaline significantly increased dP/dtmax in control and mild acidosis groups at  $10^{-7}$  M and in all groups at  $10^{-6}$  M. \* $P < 0.05$  vs. pre-pH change.  $^{\dagger}P < 0.05$  vs. post-pH change and time (vehicle) control values at corresponding pH (Figure 3).  $^{\ddagger}P < 0.05$  vs. control (pH 7.4) group. Although LV dP/dtmax significantly increased with  $10^{-6}$  M adrenaline in the severe acidosis group, it was significantly less than that of the control group.

**Figure 7:** Changes in coronary perfusion flow (CPF) with or without adrenaline at pH 7.4, 7.0 and 6.6. Data are shown as means  $\pm$  SD (n=8 each). Change in pH decreased CPF in severe acidosis group. Adrenaline significantly increased CPF in the control and mild acidosis groups at all concentrations studied, but not in the severe acidosis group. \* $P < 0.05$  vs. pre-pH change.  $^{\dagger}P < 0.05$  vs. post-pH change and time (vehicle) control values at corresponding pH.

**Figure 8:** Cyclic adenosine monophosphate (cAMP) contents in isolated left ventricular muscles perfused with or without colforsin or adrenaline at pH 7.4 and 6.6. Data are shown as means  $\pm$  SD (n=6 each). Colforsin or adrenaline was given in the identical sequence as the experiment 1 (Figure 1). Decrease in pH from 7.4 to 6.6 resulted in significantly less cAMP without cardiogenic drugs. Colforsin significantly increased cAMP contents at pH 7.4 and 6.6. Similarly, adrenaline also increased cAMP contents both at pH 7.4 and 6.6, but resulted in significant difference between pH 7.4 and 6.6 after  $10^{-6}$  M adrenaline. \* $P < 0.05$  between pH 7.4 and 6.6.  $^{\dagger}P < 0.05$  vs. without colforsin or adrenaline at corresponding pH.

Fig. 1

### Experiment 1



### Experiment 2

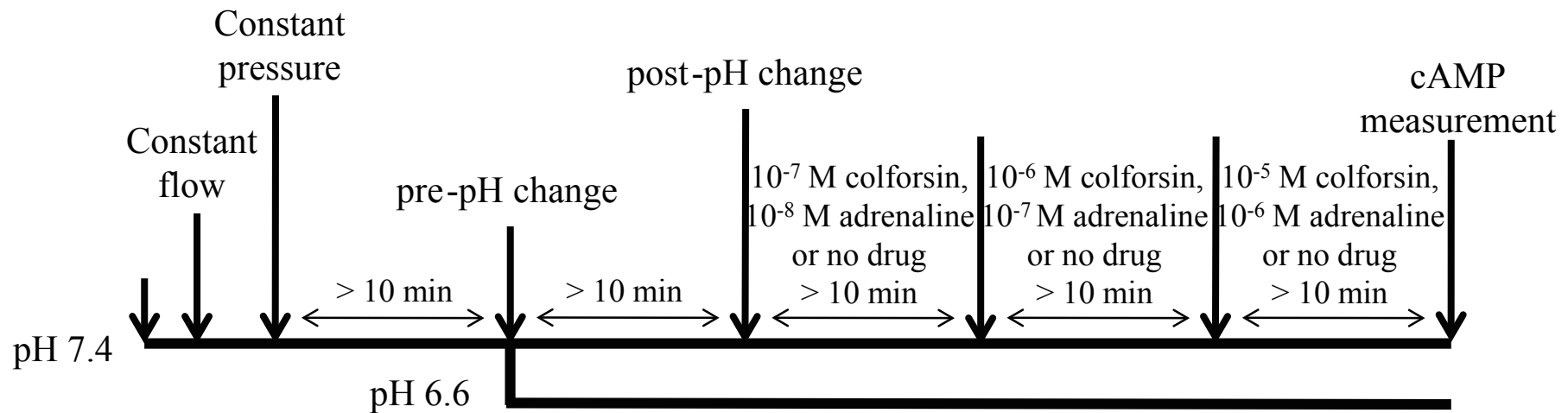




Fig. 2

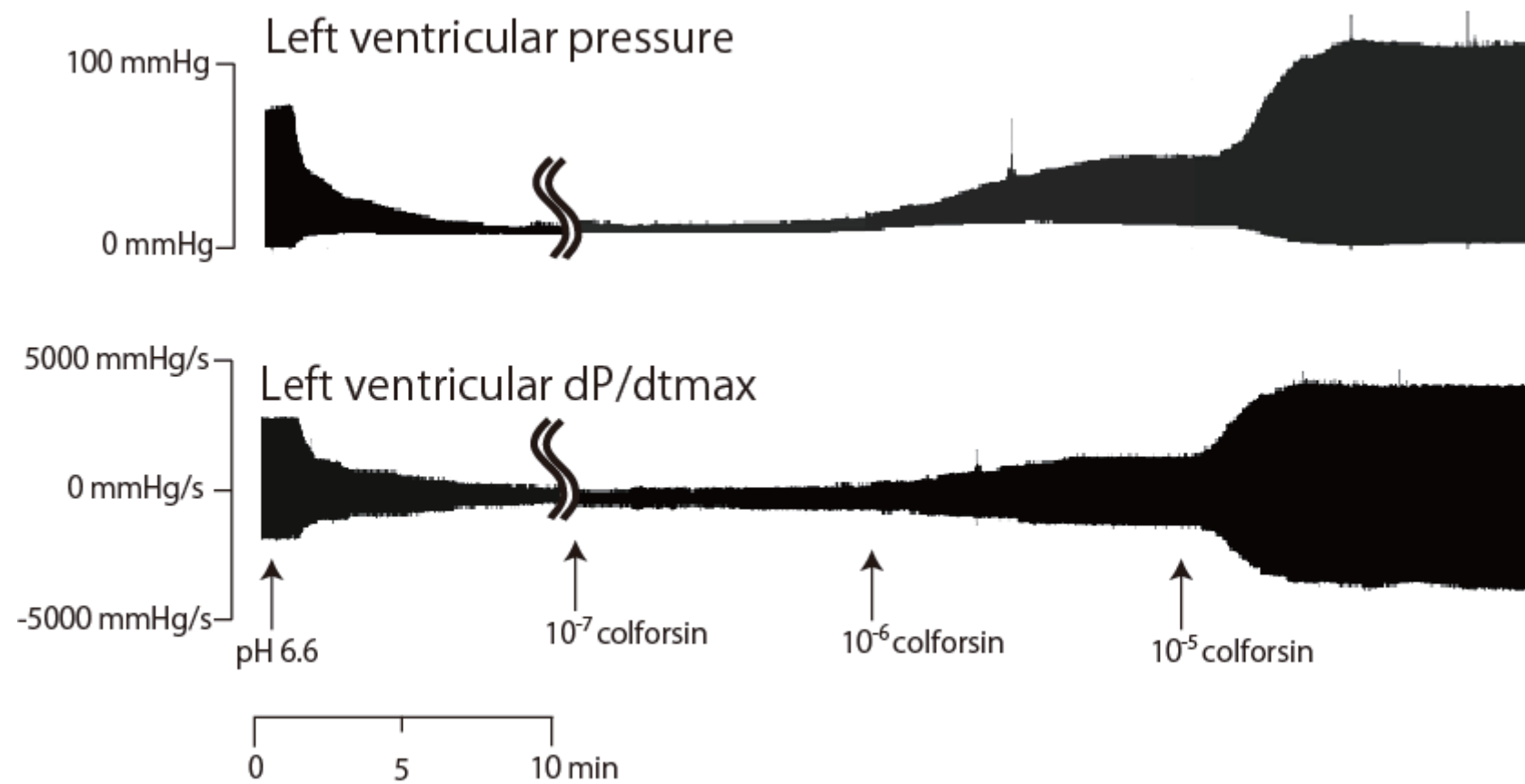


Fig. 3

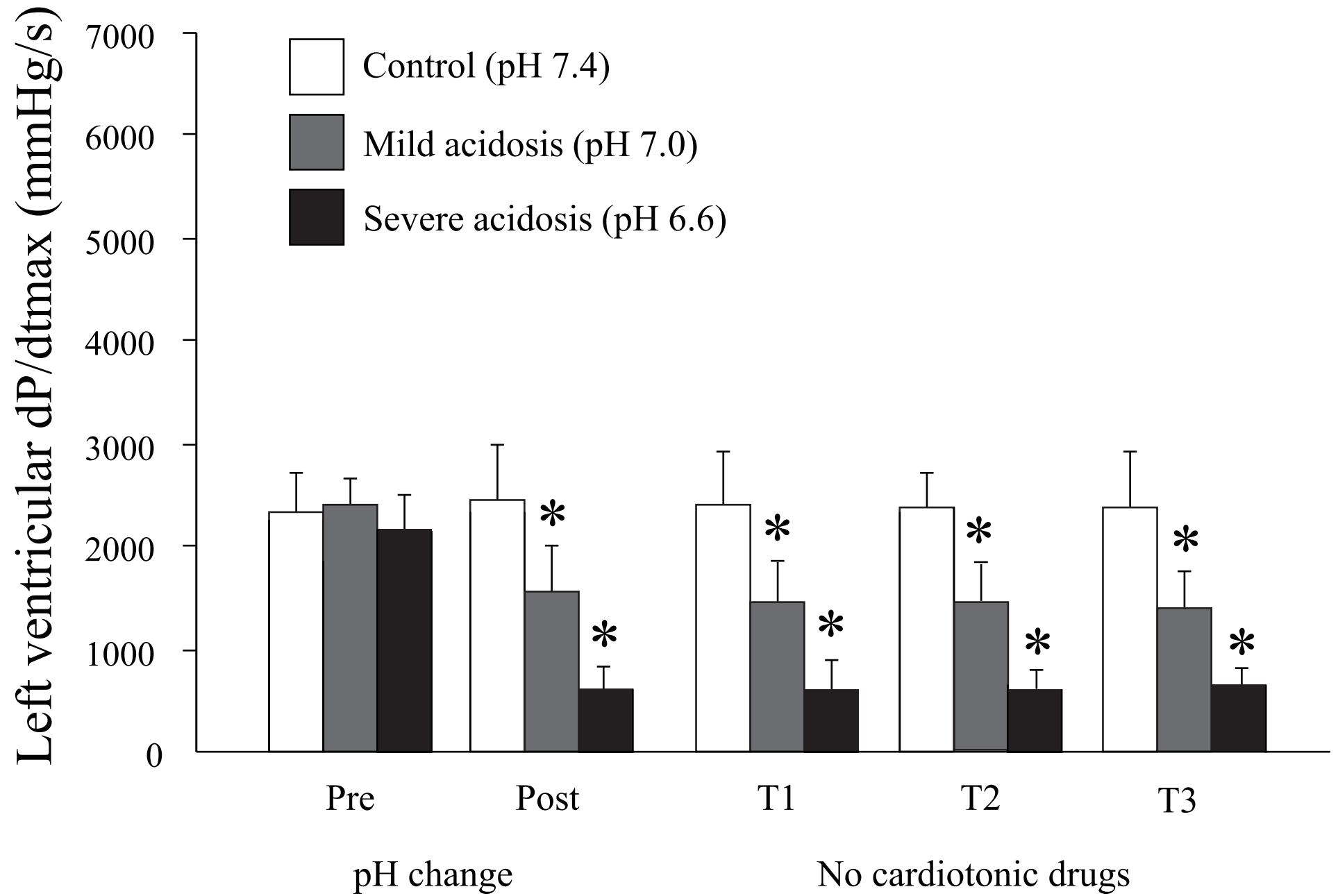


Fig. 4

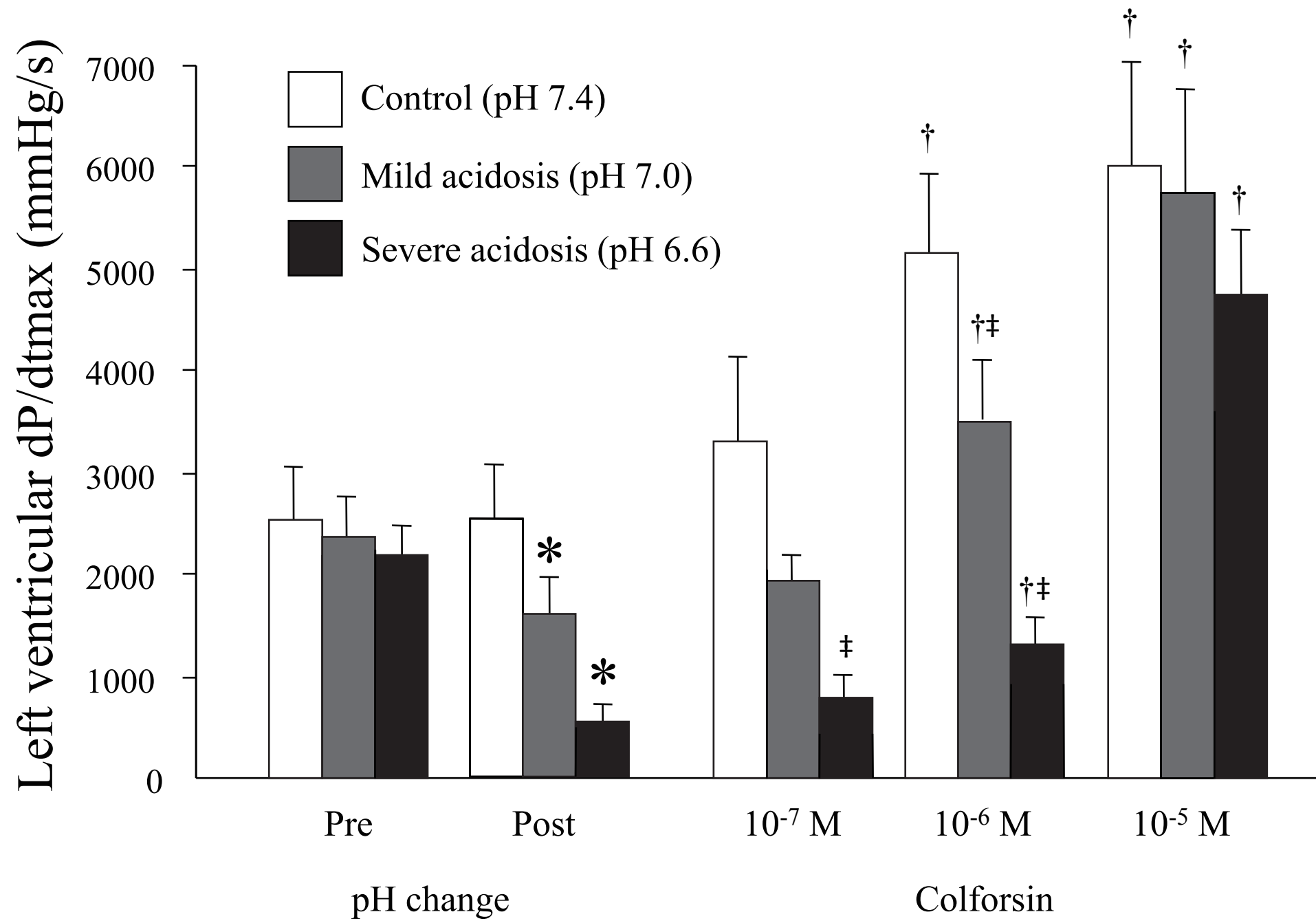


Fig. 5

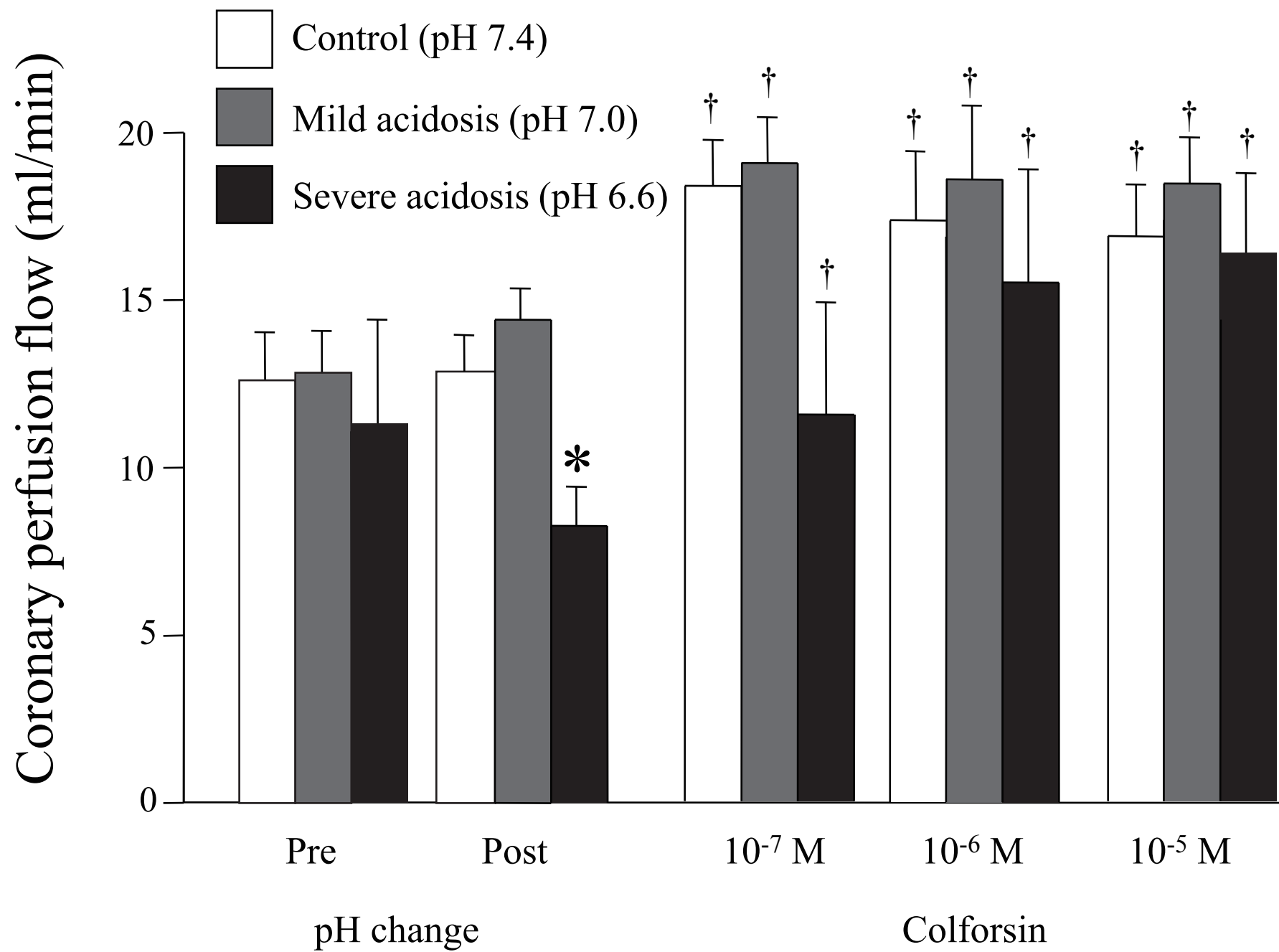


Fig. 6

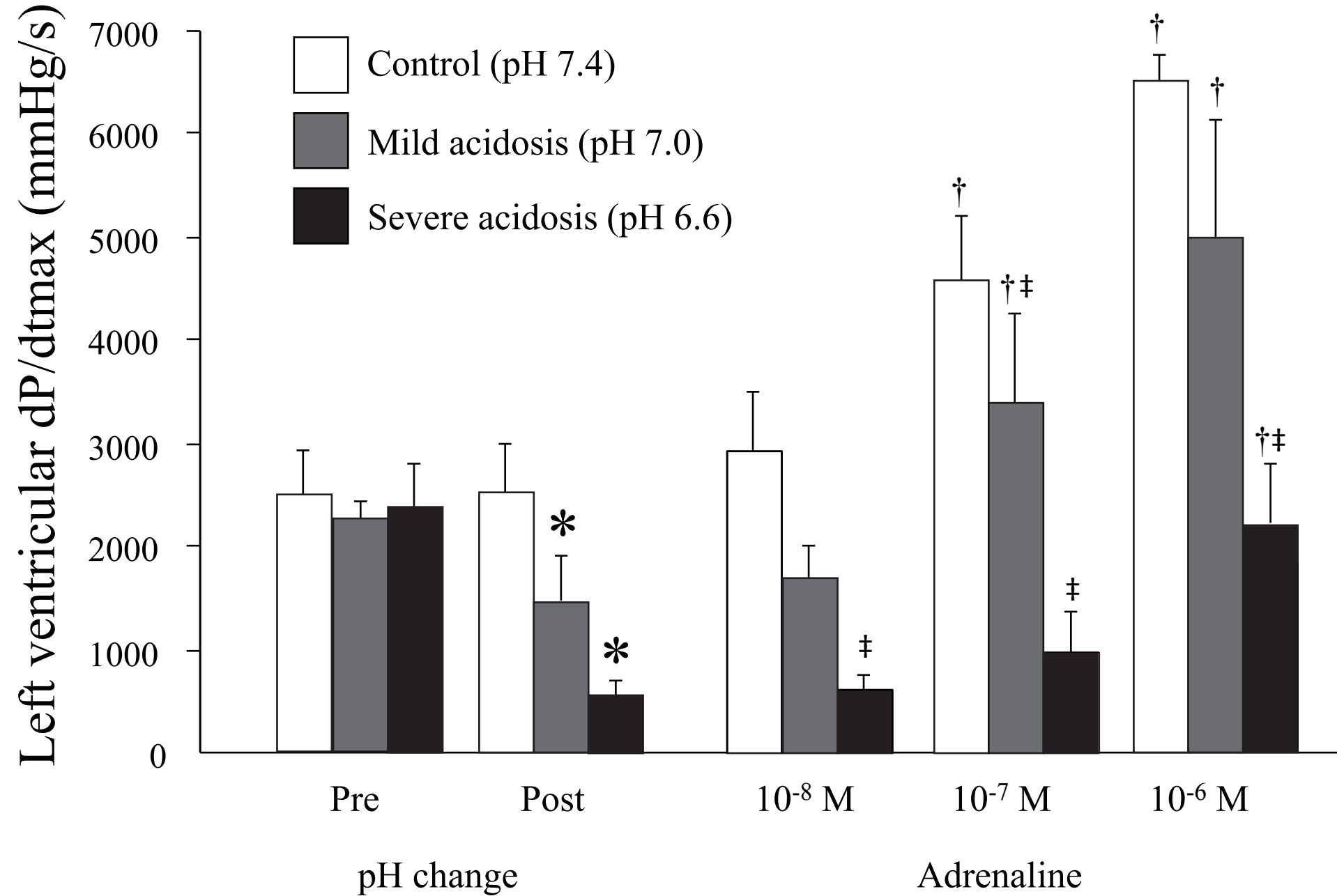


Fig. 7

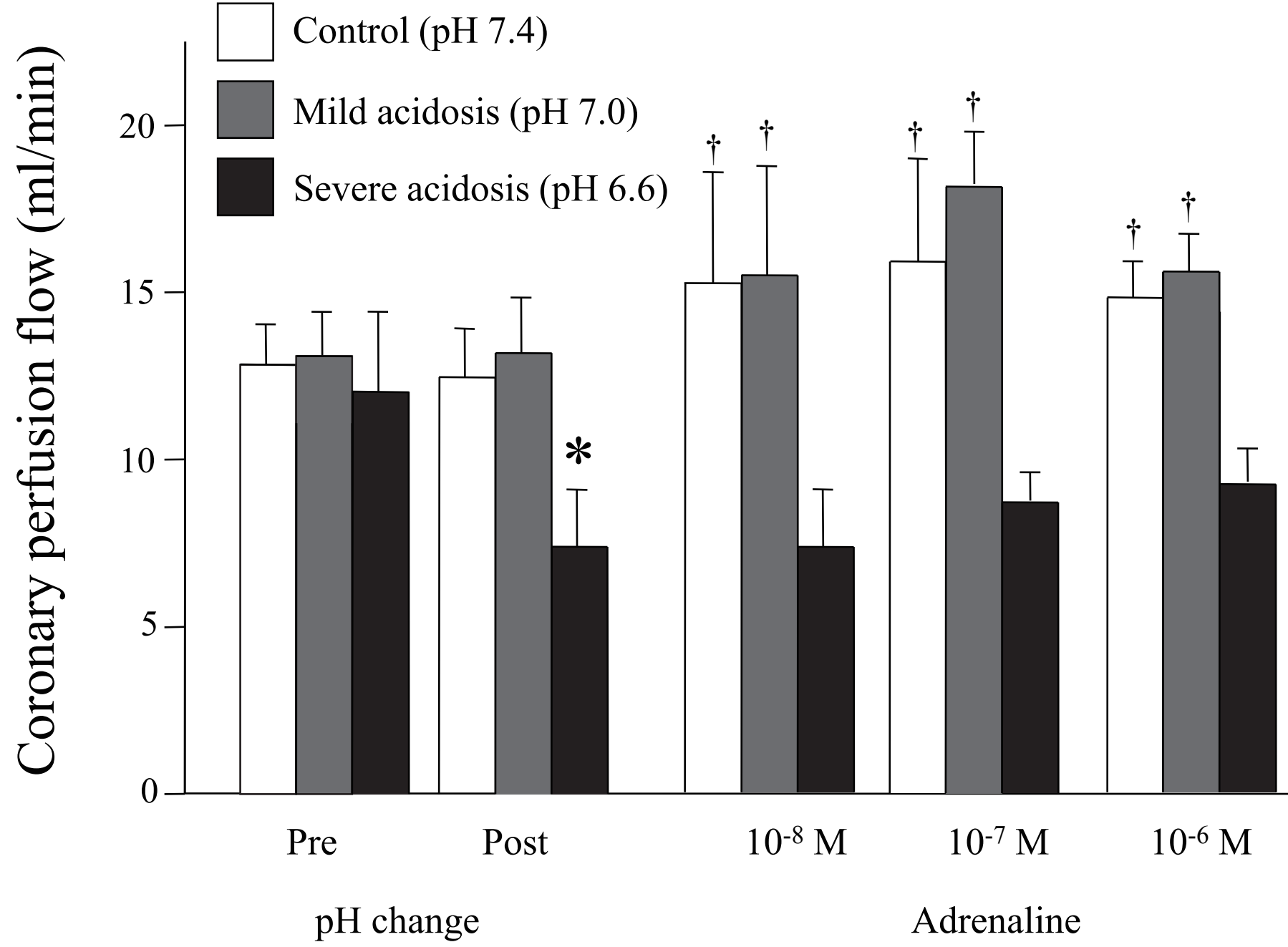


Fig. 8

